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Molecular cloning and characterization of a β-glycosidase gene from the termite *Reticulitermes flaviceps* (Isoptera: Rhinotermitidae)

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Abstract: 【Aim】*Reticulitermes flaviceps* is one of the representative wood-feeding termite in our country, which has efficient cellulolitic system. This study aims to clone and characterize cellulase gene from *R. flaviceps*, so as to provide the theoretical basis for exploring cellulose resource of termites. 【Methods】A β-glycosidase gene was cloned from *R. flaviceps* by using reverse-transcription polymerase chain reaction (RT-PCR), degenerate PCR and rapid amplification of cDNA end (RACE) methods, and its sequence was analyzed by bioinformatics tools. The corresponding protein was prokaryoticly expressed in *Escherichia coli* and the cellulase activity was measured after purification. 【Results】The full-length cDNA sequence of β-glucosidase gene *GZRfbg*1 (GenBank accession no.: KU886065) was cloned from *R. flaviceps*, with a length of 1 691 bp and an open reading frame of 1 488 bp, encoding 448 amino acid residues with the typical structure of glycosyl hydrolase family (GHF) 1 domain. The calculated protein molecular weight is 56.45 kD. Multiple alignment analysis revealed that the deduced amino acid sequence of GZRfBG1 shares 95% identity with the salivary gland β-glucosidase of *Reticulitermes flavipes*. GZRfBG1 was heterologously expressed in *E. coli* Rosetta 2 (DE3). The purified tagged GZRfBG1 exhibited increased specific activity to salicine, carboxymethyl cellulose sodium (CMC), and cellobiose. And the cellulolytic activity was also shown in a CMC containing agarose plate, in which clear digestion haloes were formed. 【Conclusion】These results suggested that the salivary gland β-glucosidase from sibling species *R. flaviceps* and *R. flavipes* are very conservative in sequence and hydrolysis characteristics. This study of β-glucosidase gene can enrich the theoretical basis for the development of termite cellulases and phylogenetic study.

Key words: *Reticulitermes flaviceps*; β-glucosidas; cDNA; heterelogous expression; salivary gland

1 INTRODUCTION

Lower wood-feeding termites, with profoundly efficient abilities to digest cellulose, play an important role in the recycling of wood lignocelluloses (Feng *et al.*, 2015; Peterson and Scharf, 2016). These termites possess a dual cellulose degradation mechanism. Endogenous cellulases and symbiotic microorganism (bacteria and protozoa) cellulases cooperatively contribute to cellulose digestion. This system has three main types of cellulases, *i.e.*, endoglucanases (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21) (Scharf *et al.*, 2011a, 20116; Brune, 2014). β-Glucosidases can hydrolyze glucose dimers and cello-oligosaccharides to glucose, and this is essential to complete cellulose digestion (Tarayre *et al.*, 2015). Lower termites can secrete endogenous β-glucosidases in their salivary glands and midguts (Watanabe, 1998; Lo and Watanabe, 2011). The salivary gland-specific β-glucosidase has been analyzed via heterologous expression systems in several termite species, including *Neotermes koshunensis* (Shiraki) (Ni *et al.*, 2007; Uchima *et al.*, 2011), *Nasutitermes takasagoensis* Shiraki (Tokuda *et al.*, 2002, 2009; Uchima *et al.*, 2013), *Coptotermes formosanus* (Shiraki) (Zhang *et al.*, 2010; Zhang *et al.*, 2012a) and *Reticulitermes flavipes* (Koller) (Hirayama *et al.*, 2010; Scharf *et al.*, 2010). Recombinant cellulolytic enzymes are useful for studying cellulose metabolism in termites and have the potential to be engineered as biocatalysts for cellulose-based biofuel production.

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The wood-feeding termite *Reticulitermes flaviceps* (Oshima) is widely distributed in southern China. The salivary gland β-glucosidase of *R. flaviceps* was reported firstly in 2008. Its nucleotide or amino acid sequence remains unknown, but its estimated molecular mass is 93.6 kD. In this study, we cloned an endogenous β-glycosidase gene named *GZRfbg*1 (glycoside hydrolase family 1 gene from the salivary gland of *R. flaviceps*). Its encoded protein has the molecular mass of ~54.6 kD. Bioinformatics analysis and heterologous expression of *GZRfbg*1 were then performed. We expect that these results will enrich our understanding of the termite lignocellulase system.

2 MATERIALS AND METHODS

2.1 Termites

Workers of *R. flaviceps* were collected from a laboratory maintained single colony and washed with double distilled water before use.

2.2 cDNA cloning of β-glucosidase gene from *R. flaviceps*

Total RNA and cDNA of the whole termite body were obtained using a Total Isolation System Kit and the PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa, Japan), respectively, using 1.2 μg RNA as a template for cDNA synthesis. To obtain cDNA fragments of the target gene, PCR was carried out using degenerate oligonucleotide primers that were synthesized based on the other identified partial amino acid sequences of β-glucosidases, and the primers used for degenerate PCR were designed by the iCODEHOP program (http://blocks.fhcrc.org/codehop.html) and shown in Table 1. The degenerate PCR reaction system of 50 μL contained each primer (2 μmol/L) 2 μL, 25 μL of Taq DNA polymerase mixture (TaKaRa, Japan). The thermal cycling conditions were 94℃ for 2 min, followed by 30 cycles of 94℃ for 30 s, 50℃ for 30 s, 72℃ for 30 s, and then 72℃ for 10 min. Based on the sequences of degenerate PCR products, primers shown in Table 2 were designed for 3′-RACE and 5′-RACE. One round 3′-RACE, three rounds 5′-RACE and nested-PCR were performed according to manufacturer's instructions (Invitrogen, GeneRacer™ Kit, USA). The amplified fragments were sequenced and assembled. The complete cDNA was amplified and cloned into a plasmid vector pMD-20T (TaKaRa, Japan). Five clones were randomly selected for sequence analysis and elimination of the PCR mutations. The final determined sequence was examined using the BLAST program provided by NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The cleavage site of the signal peptide was predicted by the SignalP program (http://www.cbs.dtu.dk/services/SignalP). Nuclear export signals were predicted using a combination of neural networks (NN) and hidden Markov models (HMM) (http://www.cbs.dtu.dk/services/NetNES/). The NetPhos 2.0 server neural network program was used to predict serine, threonine and tyrosine phosphorylation sites (http://www.cbs.dtu.dk/services/NetPhos/). Prediction of transmembrane helices in proteins was accomplished using the transmembrane helices program on the TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/).

Table 1 Degenerate primers used to amplify the β-glucosidase gene from *Reticulitermes flaviceps*

Primers	Primer sequences (5′-3′)	GenBank accession numbers of the corresponding homologous protein sequences
F1	GGAAAGGAGTTAATATHTGGGAYAC	
F2	TACCCCGGGGATCGGNGAYTAYYT	
F3	ACTTCTTTGGCATGAAYTWYTAYAC	
R1	TANATRGTRACCCTGGACGGCGT	gi269965728, gi269965726, gi269965730;
R2	ATRGTRACCCTGGACGGCGTCTTTG	gi269965724, gi269965718, gi269965720;
R3	CCNYTRATRGGAAGGCATCACTTTC	gi291293226, gi303324839, gi303324840;
R4	CTYCCNAWRTGATGGAGAGCCGAT	gi300432455, gi283484500
R5	TTRACCTADYKTTTGCTCATACCGT	
R6	ATRATRTGNCTCGTGTACTTCCTCT	
R7	CTRTTRAARCTCACCGACGCTCCC	

Degenerate nucleotides are as follows: R = A/G, Y = C/T, M = A/C, K = G/T, S = C/G, W = A/T, H = A/C/T, B = C/G/T, V = A/C/G, D = A/G/T, N = A/C/G/T.

Table 2 Primers used in RACE

Primers	Primer sequences (5′ – 3′)	Use of primers
R. B1B7-3′Race-sp1	CGGAATTCCAGTGTTTTGAAAGCGAGGGTAGAC	3′-RACE
R. B1B7-3′Race-sp2	CGGAATTCGCCTGGATTGAGCCGGTGACTAA	
AP	GGCCACGCGTCGACTAGTACT TTTTTTTTTTTTTTTT	
AUAP	GGCCACGCGTCGACTAGTAC	
R. B1B7-5′Race-sp1	CGGAATCCCCATGGTACAACTCTG	5′-RACE Round 1
R. B1B7-5′Race-sp2	TGAATTTTGGTAGACGGGATGTGG	
5′Race abridged[a]	GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG	
5′Race abridged anchor	GGCCACGCGTCGACTAGTAC	
R. B1B7-5′Race-sp4	CTCGCTTTCAAAACACTGGGGTAA	5′-RACE Round 2
B1B7-5′Race-sp5	AACCTCTCCTTTCTGATCCGCTTTGA	
B1B7-5′Race-sp6	GTAACCATCGGCTCTATTTCATTGTC	5′-RACE Round 3

[a] The "II" in 5′-RACE abridged primer represents poly "G".

2.3 Expression of recombinant protein in *Escherichia coli*

The sequence of β-glucosidase gene, without the nucleotide sequences encoding the putative N-terminal signal peptide, was amplified by PCR and analyzed by sequencing. The PCR primer sequences were as follows: the forward primer was flanked at the 5′ end with an *Nco*I restriction sequence (5′-CCATGGGACGTCGATAACGAAACCCT-3′) and the reverse primer flanked at the 5′ end with an *Xho*I restriction sequence omitting the stop codon (5′-CTCGAGGTCTAGGAAGCGTTCTGGAAT-3′) for His-tag fusion expression. The underlined was used to indicate the recognition site of restriction enzyme. The PCR reaction system of 50 μL contained a final concentration of 0.1 μmol/L of each primer, DNA templates at a final concentration of 100 ng, and 25 μL of Taq DNA polymerase Mixture (TaKaRa, Japan). The thermal cycling conditions were 94℃ for 2 min, followed by 30 cycles of 94℃ for 30 s, 55℃ for 30 s, 72℃ for 1 min, and then 72℃ for 10 min. The cellulase gene overlapped with *Nco* I and *Xho* I cohesive ends was cloned into pET32a vector (Novagen, USA) at corresponding restriction sites. Recombinant pET32a plasmids were propagated in *Escherichia coli* DH5α and five clones were randomly selected for sequencing verification. Competent cells of *E. coli* BL21 and Rosetta 2 (DE3) (Novagen, USA) were transformed individually with the following 2 plasmid constructs: (1) recombinant pET32a plasmid carrying the cellulase gene without the stop codon at the 3′ end, thereby allowing the expression of recombinants with 6 × His residues downstream of the gene (referred to as t*GZRfbg*1 hereafter), and (2) original control pET32a plasmid with no cleavage or insertions (referred to as pET32a-CK hereafter). Resulting colonies were randomly picked for growth in Luria-Bertani (LB) medium with ampicillin (100 μg/mL) in shaker flask. *E. coli* cells were incubated at 37℃ at 220 r/min and induced with 0.5 mmol/L isopropyl β-D-1-thiogalactopyranoside (IPTG) (Dingguo Changsheng, China) for an additional 3 h when the optimal density reached 0.4 – 0.5 at 600 nm. Induced *E. coli* cells were divided into two aliquots (A and B) and harvested by centrifugation: (A) the cell pellet was suspended in 50 mmol/L Tris-HCl buffer (pH 8.0) containing 5 mmol/L EDTA and 1 mmol/L PMSF (Dingguo Changsheng, China). The cells were lysed by sonication in ice-water and centrifuged at 8 000 × *g* at 4℃ for 20 min. The supernatant and the insoluble matter fractions were boiled for 5 min after adding 2 × SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) loading buffer to detect soluble protein and proteins expressed as inclusion bodies (IB), respectively; (B) the cell pellet was suspended in 2 × SDS-PAGE loading buffer and boiled for 5 min then used for analysis of total cell protein (TCP). SDS-PAGE and Western blot were used to identify the expression of t*GZRfbg*1 and carried out referring to Molecular Cloning III (Sambrook, 2001). The protein bands were blotted onto a polyvinylidene difluoride (PVDF) membrane and successively incubated with primary antibody (mouse-anti-His-tag) and HRP-conjugated anti-mouse IgG (MBL, Japan). The immunoreactive bands were visualized with a DAB (3, 3′ N-diaminobenzidine tertrahydrochloride) Western Blotting Detection Kit (CWBIO, China).

2.4 Isolation and purification of recombinant protein

For large-scale isolation and purification of recombinant protein, recombinant *E. coli* Rosetta 2 (DE3) cells were grown in 100 mL LB with antibiotics as described in section 2.3 and shake-incubated at 37℃ for 2 – 3 h at 220 r/min and induced with 0.1 mmol/L IPTG for an additional 18 h at 20℃ when the optimal density reached 0.4 at 600 nm. The cells were harvested by centrifugation and lysed by the sonication method described in section 2.3. The supernatant was recovered by centrifugation at 5 000 × *g* at 4℃ for 20 min and the cell debris was removed by passage through a membrane filter ($\Phi = 0.45$ μm, Millipore, USA). The purification of soluble tGZRfBG1 was performed according to the manufacturer's instructions using a 6 × His-tagged (Ni-Agarose) Soluble Protein Purification Kit (CWBIO, China). Following purification, the purified protein was stored in 100 mmol/L sodium acetate (SAB), pH 5.6, with 10% glycerol, using an Ultra-15K centrifugal filter unit (Millipore, Cork, Ireland). Purified tGZRfBG1 was preserved in SAB (pH 5.6) with 30% glycerol. Protein concentration was estimated with the Bradford Reagent (CWBIO, China) using bovine serum albumin (BSA) as the standard protein. SDS-PAGE and Western blotting were used to identify the purified tGZRfBG1 and performed as previously described in section 2.3.

2.5 Activity assays of recombinant cellulase

The pET32a-CK, expressed and purified under the same conditions as tGZRfBG1 in section 2.4, was used as the negative control protein. The cellulolytic activity of purified soluble tGZRfBG1 was evaluated by the following assays: (1) Agarose plate assay: the agarose plate was made with 1% agarose and 0.2% carboxymethyl cellulose sodium (CMC) in distilled water. The purified tGZRfBG1 and pET32a-CK solutions were spotted on the surface of the plate in equal amounts. The plate was then incubated at 37℃ for 18 h. The enzyme activity was visualized using the Congo Red Stain method (Zhang *et al.*, 2009). (2) Substrate specificity assay: the enzyme was incubated with 1% CMC, 1% salicine, and 1% cellobiose, respectively. Enzyme activity was assayed by measuring the release of glucose from this substrate using the dinitrosalicylic acid (DNS) method (Zeng, 2016). Each assay was performed in triplicate. One unit (U) of specific enzyme activity was defined as the amount (μmol) of reducing sugars (glucose equivalent) produced by 1 mg of protein per min. An equal amount of pET32a-CK solution was used as the negative control.

3 RESULTS

3.1 Sequence features of β-glucosidase gene of *R. flavicepes*

The full-length cDNA of β-glucosidase gene of *R. flavicepes* obtained in this study was 1 691 bp and designated as *GZRfbg*1 (GenBank accession no.: KU886065). This cDNA contains an open reading frame (ORF) of 1 488 bp. The translation initiation codon ATG in cDNA nucleotide position is from 45 to 47 bp and the termination codon TAA is from 1 530 to 1 532 bp. In the 3′-terminal region, there is a putative poly-adenylation signal sequence AATAAA and a poly (A+) tail, suggesting that the *GZRfbg*1 was derived from an eukaryotic organism (Fig. 1). The ORF encodes 448 amino acids with the calculated molecular weight of 56.45 kD. This multiple alignment results showed that GZRfBG1 has the typical structure of glycosyl hydrolase family (GHF) 1 domain and shares 95%, 87%, 86%, 83% and 81% amino acid sequence identity with the salivary gland β-glucosidase sequences of *R. flavipes* (GenBank accession no.: ADK12988.1), *Coptotermes gestroi* (GenBank accession no.: AGS32242.1), *C. formosanus* (GenBank accession no.: ADB23476.1), *Macrotermes barneyi* Light (GenBank accession no.: AFD33364.1) and *Nasutitermes takasagoensis* (GenBank accession no.: BAI50023.1), respectively (Fig. 2). A signal peptide cleavage site between the 17th and 18th amino acid residue was found. Generic phosphorylation sites analysis results showed that GZRfBG1 has 12 serine, 4 threonine and 9 tyrosine phosphorylation sites. A nuclear export signal (NES) was detected at the 430th amino acid residue. Transmembrane helices analysis indicated that GZRfBG1 is probably located outside the membrane. All the forecasting mentioned above suggested that GZRfBG1 has post-translational modification and can be secreted from cells into the surrounding tissues.

3.2 Expression and purification of the recombinant GZRfBG1

Over-expressions of t*GZRfbg*1 and pET-32a vector (pET32a-CK) were detected by IPTG induction of LB culture, and analyzed by SDS-PAGE and Western blot. The expression of tGZRfBG1 was not detected in *E. coli* BL21 cells. Most tGZRfBG1 was expressed as inclusion bodies in *E. coli* Rosetta 2 (DE3) cells. The expected expressed fusion protein encoded by pET32a-CK alone would be approximately 26 kD. The tGZRfBG1 was expressed with Trx. tag, S. tag and His-tag. In comparison with the protein profile of pET32a-CK (negative control), a distinctive band with calculated molecular weight (MW) around 74 kD was found in

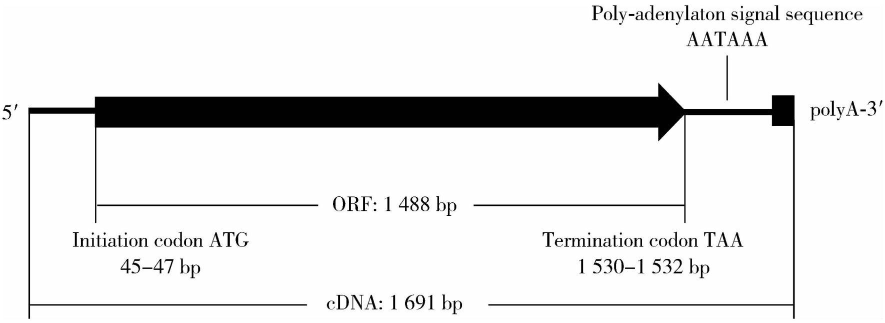

Fig. 1　Schematic structure of *GZRfbg*1 cDNA of *Reticulitermes flaviceps*

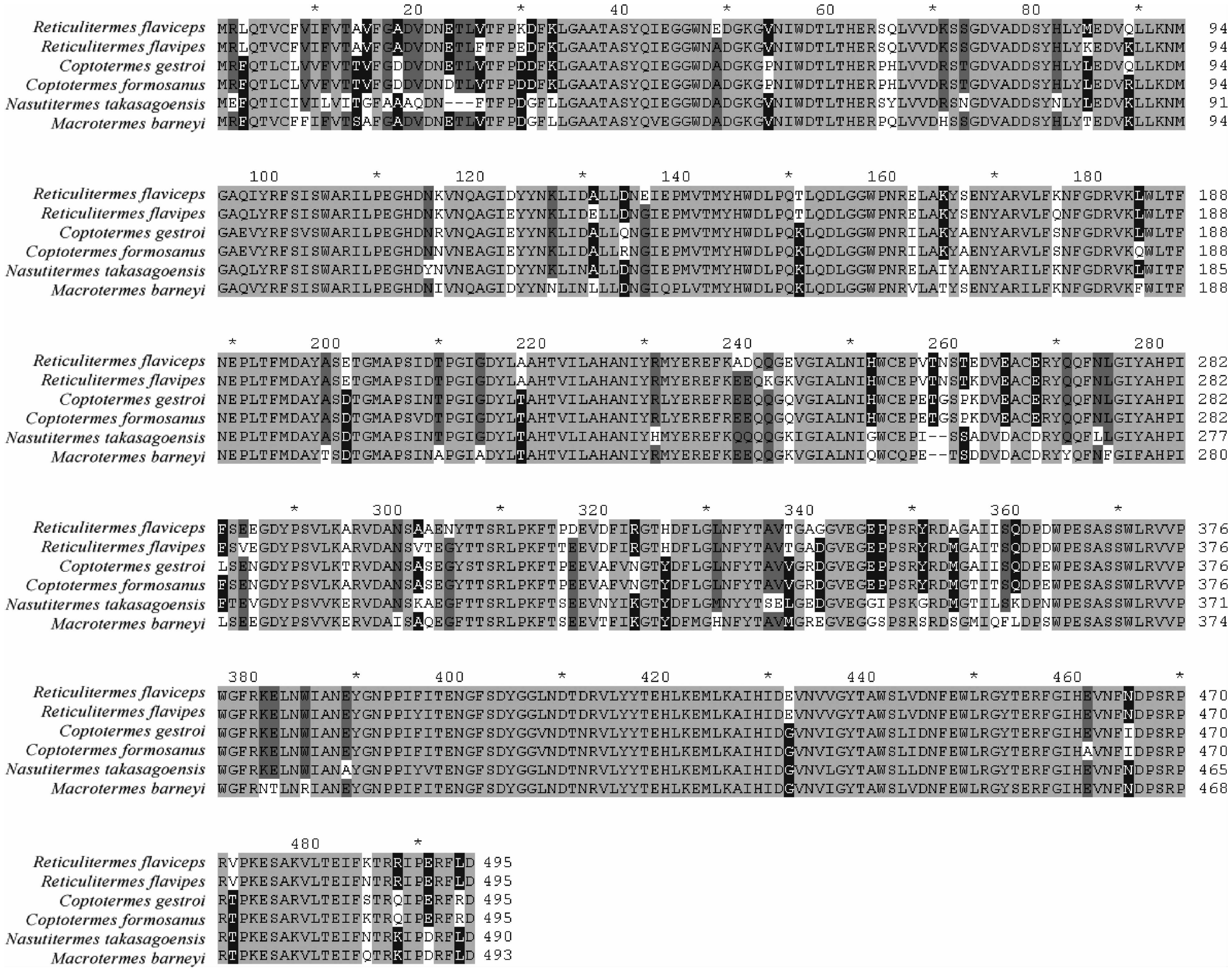

Fig. 2　Multiple alignments of amino acid sequences of the β-glucosidase genes of *Reticulitermes flaviceps* (GZRfBG1) and other termite species

Species source and GenBank accession numbers of β-glucosidase genes: *Reticulitermes flaviceps* (GZRfBG1), AMY26906.1; *Reticulitermes flavipes*, ADK12988.1; *Coptotermes gestroi*, AGS32242.1; *Coptotermes formosanus*, ADB23476.1; *Nasutitermes takasagoensis*, BAI50023.1; *Macrotermes barneyi*, AFD33364.1. The black, dark gray and light gray denote the amino acid sequence identity of 60%, 80% and 100%, respectively.

the tGZRfBG1 inclusion body, as well as soluble protein in cell lysed supernatant, and purified soluble tGZRfBG1. During ultrafiltration of tGZRfBG1, precipitate was observed at a high protein concentration. The final concentrations of purified tGZRfBG1 and pET32a-CK in 100 mmol/L SAB (pH 5.6, 30% glycerol) were 510 and 1 080 μg/mL, respectively (Fig. 3).

3.3　Enzymatic activity of the recombinant β-glucosidase

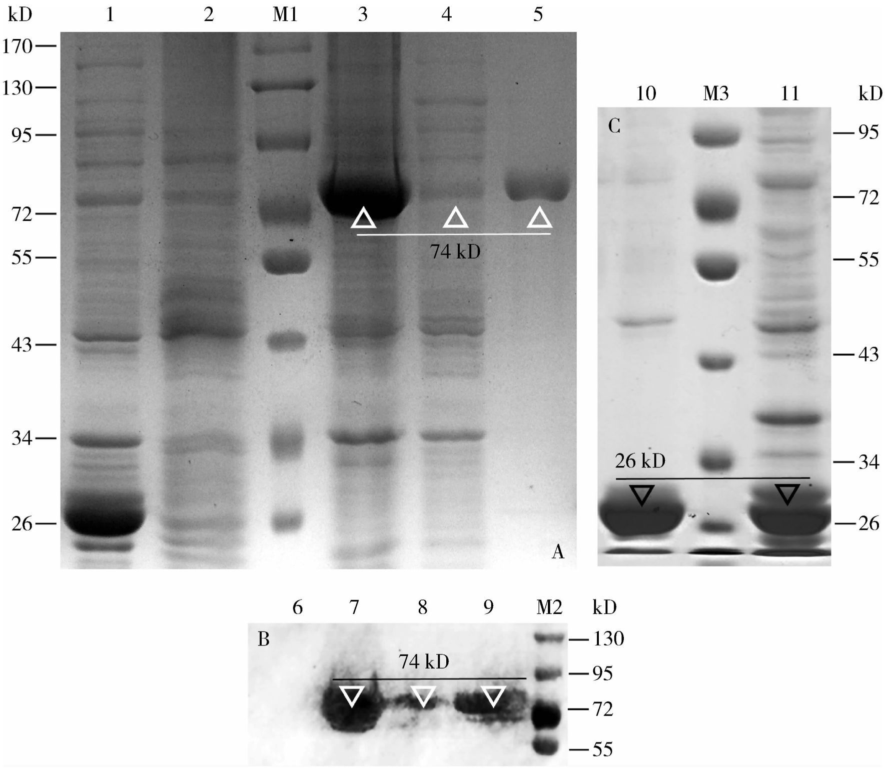

Fig. 3 SDS-PAGE (A, C) and Western blot (B) analysis of the recombinant GZRfBG1

M1, M2, M3: Protein molecular weight marker; 1: Total protein of pET32a-CK expressed in *E. coli* Rosetta 2 (DE3); 2: Total protein of t*GZRfbg*1-pET32a plasmid expressed in *E. coli* BL21; 3: Total protein of tGZRfBG1 expressed in *E. coli* Rosetta 2 (DE3); 4: Supernatant of crude cell lysate of tGZRfBG1; 5: Purified soluble tGZRfBG1; 6: Total protein of pET32a-CK expressed in *E. coli* Rosetta 2 (DE3); 7: Total protein of tGZRfBG1 expressed in *E. coli* Rosetta 2 (DE3); 8: Supernatant of crude cell lysate of tGZRfBG1; 9: Purified soluble tGZRfBG1; 10: Purified soluble pET32a-CK; 11: Total proteins of pET32a-CK expressed in *E. coli* Rosetta 2 (DE3).

The pET32a-CK solution was used as a negative control. The cellulolytic activity of purified tGZRfBG1 was observed in the CMC-containing agarose plate. The halo diameters formed by 2.04 (4 μL) and 4.08 μg (8 μL) purified tGZRfBG1 were 5 and 9 mm, respectively. No halo was formed by equivalent amounts of the pET32a-CK solution (Fig. 4). The specific activity of purified tGZRfBG1 to cellobiose, CMC and salicine, measured by reducing sugar production and expressed as glucose equivalent, was 0.609 ± 1.27E-01, 0.087 ± 3.41E-02 and 0.009 ± 1.74E-03 U/mg, respectively (Fig. 5). Errors were given as standard deviation of three independent experiments.

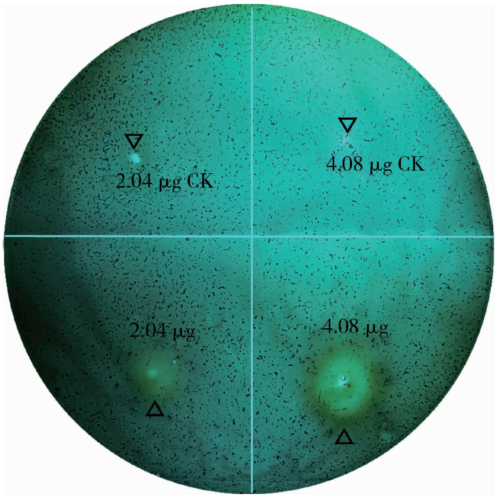

Fig. 4 Enzymatic activity of purified tGZRfBG1 on an agarose plate containing 0.2% CMC

Equal amounts (μg) of individual tGZRfBG1 and pET32a-CK preparations were spotted on the plate. CK indicates the negative control pET32a-CK. The plate was incubated at 37℃ for 18 h and the halos, indicating activity, were shown after staining with 0.1% Congo red and de-stained with 1 mol/L NaCl.

4 DISCUSSION

Termites are the key cellulose consumers and digesters in natural ecosystem, and they have the outstanding ability to degrade lignocelluloses using their unique enzyme complexes (Sun and Scharf, 2010; Sun, 2014). *R. flaviceps* is one of a representative wood-feeding termite species in China. A full-length cDNA sequence of β-glucosidase designated as *GZRfbg*1 was cloned from

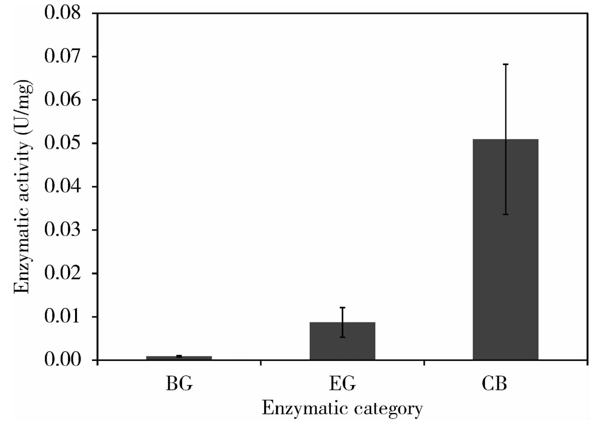

Fig. 5　Enzymatic activity of purified tGZRfBG1 toward different substrates

The specific activity of purified tGZRfBG1 toward cellobiose (CB), CMC (EG) and salicine (BG) was measured by reducing sugar production and expressed as glucose equivalent. Each value is the mean ± *SD* of three independent experiments.

R. flaviceps and heterologously expressed in *E. coli* Rosetta 2 (DE3). Understanding the cellulolytic enzymes adopted by termites may help biomass utilization projects.

In 2008, Xue *et al.* (2008) firstly purified a protein from the salivary gland of *R. flaviceps* with β-glucosidase activity. Its nucleotide or amino acid sequence remains unknown, but its predicated molecular mass is 93.6 kD which is much larger than that of GZRfBG1 (56.45 kD). Multiple endogenous β-glucosidases with different properties and functions had been found in *C. formosanus* and *Reticulitermes speratus* Kolb (Shimada and Maekawa, 2014; Feng *et al.*, 2015), suggesting that different endogenous β-glucosidases maybe also encoded by *R. flaviceps*. Based on the amino acid sequence, GZRfBG1 can be categorized as a GHF1 enzyme. It has a digestive function similar to other endogenous termite β-glucosidases, including RfBGluc-1 from *R. flavipes* (Scharf *et al.*, 2010; Zhang *et al.*, 2012b), MbEG1 from *Macrotermes barneyi* (Wu *et al.*, 2012), G1NkBG from *N. koshunensis* (Uchima *et al.*, 2011), CfBGI from *C. formosanus* (Zhang *et al.*, 2010; Zhang *et al.*, 2012a). Purified tGZRfBG1 exhibited increased specific activity toward salicine, CMC and cellobiose, and also formed hydrolyzation circles on CMC-agarose plates. CMC is normally used as the substrate for EG activity measurement and salicine is typically used as the substrate for measurement of BG activity (Eveleigh *et al.*, 2009). The RfBGluc-1 also showed relatively higher activity to cellobiose than to other substrates. And the activity of β-glucosidase protein of 93.6 kD purified from *R. flaviceps* exhibited similar optimum temperature, pH and substrate preference to RfBGluc-1 (Xue *et al.*, 2008; Scharf *et al.*, 2010). Multiple alignment analysis revealed that the deduced amino acid sequence of GZRfBG1 shares 95% identity with RfBGluc-1. These results suggested that the salivary gland β-glucosidase from sibling species *R. flaviceps* and *R. flavipes* are very conservative in sequence and hydrolysis characteristics.

The low β-glycosidase activity of tGZRfBG1 might because that the insect originated cellulases expressed in *E. coli* could not undergo the complete processing or posttranslational modification that occurs in eukaryotic systems. Bioinformatics analysis suggested that GZRfBG1 has post-translational modification allowing secretion into tissues. The *E. coli* expression system used here was convenient and provided comparability with other research (Ni *et al.*, 2007; Wu *et al.*, 2012). But mass production of native endogenous cellulases from termites in *E. coli* can be difficult (Ni *et al.*, 2005; Hirayama *et al.*, 2010; Ni *et al.*, 2014). In this study, tGZRfBG1 was not expressed in *E. coli* BL21 but successfully expressed in *E. coli* Rosetta 2 DE3 which is specialized for eukaryotic minor codon preference. In addition, the IPTG concentration and expression temperature were optimized to increase the solubility of tGZRfBG1 (data not shown). However, most of the protein tGZRfBG1 was expressed as an inclusion body and precipitated during ultrafiltration centrifugation. So, the concentration of purified tGZRfBG1 was limited and this could be another reason for the very low cellulase activity detected in purified tGZRfBG1. Differences in the expression system or in the expression condition can have significant effects on GZRfBG1 expression and purification. Many endogenously produced termite β-glucosidase in eukaryotic expression systems (*P. pastoris*, *Aspergillus oryzae*, and baculovirus) were more thermostable or active than those expressed in *E. coli* (Ni *et al.*, 2007; Scharf *et al.*, 2010; Uchima *et al.*, 2011; Wu *et al.*, 2012). As the next step, additional properties of GZRfBG1 will be studied using a eukaryotic expression system.

This study characterized a β-glucosidase from *R. flaviceps*, which can provide a theoretical basis for the development and phylogenetic study of termite cellulases in the future.

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References

Brune A, 2014. Symbiotic digestion of lignocellulose in termite guts. *Nat. Rev. Microbiol.*, 12(3): 168-180.

Eveleigh DE, Mandels M, Andreotti R, Roche C, 2009. Measurement of saccharifying cellulase. *Biotechnol. Biofuels*, 2: 21.

Feng T, Liu H, Xu Q, Sun J, Shi H, 2015. Identification and characterization of two endogenous beta-glucosidases from the termite *Coptotermes formosanus*. *Appl. Biochem. Biotechnol.*, 176(7): 2039-2052.

Hirayama K, Watanabe H, Tokuda G, Kitamoto K, Arioka M, 2010. Purification and characterization of termite endogenous beta-1,4-endoglucanases produced in *Aspergillus oryzae*. *Biosci. Biotechnol. Biochem.*, 74(8): 1680-1686.

Lo N, Tokuda G, Watanabe H, 2011. Evolution and function of endogenous termite cellulases. In: Bignell DE, Roinsin Y, Lo N eds. Biology of Termites: A Modern Synthesis. Springer, The Netherlands. 51-67.

Ni J, Takehara M, Watanabe H, 2005. Heterologous overexpression of a mutant termite cellulase gene in *Escherichia coli* by DNA shuffling of four orthologous parental cDNAs. *Biosci. Biotechnol. Biochem.*, 69(9): 1711-1720.

Ni JF, Tokuda G, Takehara M, Watanabe H, 2007. Heterologous expression and enzymatic characterization of beta-glucosidase from the drywood-eating termite, *Neotermes koshunensis*. *Appl. Entomol. Zool.*, 42(3): 457-463.

Ni JF, Wu Y, Yun C, Yu ML, Shen YL, 2014. cDNA cloning and heterologous expression of an endo-beta-1,4-glucanase from the fungus-growing termite *Macrotermes barneyi*. *Arch. Insect. Biochem.*, 86(3): 151-164.

Peterson BF, Scharf ME, 2016. Lower termite associations with microbes: synergy, protection, and interplay. *Front. Microbiol*, 7: e6577.

Sambrook J, Russell DW, 2001. Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press, New York.

Scharf ME, Karl ZJ, Sethi A, Boucias DG, 2011a. Multiple levels of synergistic collaboration in termite lignocellulose digestion. *PLoS ONE*, 6(7): e21709.

Scharf ME, Karl ZJ, Sethi A, Sen R, Raychoudhury R, Boucias DG, 2011b. Defining host-symbiont collaboration in termite lignocellulose digestion. *Commun. Integr. Biol.*, 4(6): 761-763.

Scharf ME, Kovaleva ES, Jadhao S, Campbell JH, Buchman GW, Boucias DG, 2010. Functional and translational analyses of a beta-glucosidase gene (glycosyl hydrolase family 1) isolated from the gut of the lower termite *Reticulitermes flavipes*. *Insect Biochem. Molec. Biol.*, 40(8): 611-620.

Shimada K, Maekawa K, 2014. Gene expression and molecular phylogenetic analyses of beta-glucosidase in the termite *Reticulitermes speratus* (Isoptera: Rhinotermitidae). *J. Insect Physiol.*, 65: 63-69.

Sun JZ, 2014. Chapter 11: Lignocellulolytic systems of insects and their potential for viable biofuels. In: Sun JZ, Ding SY, Doran-Peterson J eds. Biological Conversion of Biomass for Fuels and Chemicals: Exploration of Natural Utilization Systems. Royal Society of Chemistry, London. 195-222.

Sun JZ, Scharf ME, 2010. Exploring and integrating cellulolytic systems of insects to advance biofuel technology. *Insect Sci.*, 17(3): 163-165.

Tarayre C, Bauwens J, Brasseur C, Matteotti C, Millet C, Guiot PA, Destain J, Vandenbol M, Portetelle D, De Pauw E, Haubruge E, Francis F, Thonart P, 2015. Isolation and cultivation of xylanolytic and cellulolytic *Sarocladium kiliense* and *Trichoderma virens* from the gut of the termite *Reticulitermes santonensis*. *Environ. Sci. Pollut. Res. Int.*, 22(6): 4369-4382.

Tokuda G, Miyagi M, Makiya H, Watanabe H, Arakawa G, 2009. Digestive beta-glucosidases from the wood-feeding higher termite, *Nasutitermes takasagoensis*: intestinal distribution, molecular characterization, and alteration in sites of expression. *Insect Biochem. Molec. Biol.*, 39(12): 931-937.

Tokuda G, Saito H, Watanabe H, 2002. A digestive beta-glucosidase from the salivary glands of the termite, *Neotermes koshunensis* (Shiraki): distribution, characterization and isolation of its precursor cDNA by 5′- and 3′-RACE amplifications with degenerate primers. *Insect Biochem. Molec. Biol.*, 32(12): 1681-1689.

Uchima CA, Tokuda G, Watanabe H, Kitamoto K, Arioka M, 2011. Heterologous expression and characterization of a glucose-stimulated beta-glucosidase from the termite *Neotermes koshunensis* in *Aspergillus oryzae*. *Appl. Microbiol. Biotechnol.*, 89(6): 1761-171.

Uchima CA, Tokuda G, Watanabe H, Kitamoto K, Arioka M, 2013. A novel glucose-tolerant beta-glucosidase from the salivary gland of the termite *Nasutitermes takasagoensis*. *J. Gener. Appl. Microbiol.*, 59(2): 141-145.

Watanabe H, Noda H, Tokuda G, Lo N, 1998. A cellulase gene of termite origin. *Nature*, 394(6691): 330-331.

Wu Y, Chi S, Yun C, Shen Y, Tokuda G, Ni J, 2012. Molecular cloning and characterization of an endogenous digestive beta-glucosidase from the midgut of the fungus-growing termite *Macrotermes barneyi*. *Insect Mol. Biol.*, 21(6): 604-614.

Xue YP, Jin LQ, Liu ZQ, Zhang JF, Zheng YG, 2008. Purification and characterization of beta-glucosidase from *Reticulitermes flaviceps* and its inhibition by valienamine and validamine. *Afr. J. Biotechnol.*, 7(24): 4595-4601.

Zeng WH, Li, ZQ, Liu BR, Li QJ, Zhong JH, 2016. Correlation between rearing temperature and the dual cellulolytic system of *Coptotermes formosanus* Shiraki and its intestinal microenvironment. *J. Asia-Pac. Entomol.*, 19(1): 209-216.

Zhang D, Allen AB, Lax AR, 2012a. Functional analyses of the digestive beta-glucosidase of Formosan subterranean termites (*Coptotermes formosanus*). *J. Insect Physiol.*, 58(1): 205-210.

Zhang D, Lax AR, Henrissat B, Coutinho P, Katiya N, Nierman WC, Fedorova N, 2012b. Carbohydrate-active enzymes revealed in *Coptotermes formosanus* (Isoptera: Rhinotermitidae) transcriptome. *Insect Mol. Biol.*, 21(2): 235-245.

Zhang D, Lax AR, Raina AK, Bland JM, 2009. Differential cellulolytic activity of native-form and C-terminal tagged-form cellulase derived from *Coptotermes formosanus* and expressed in *E. coli*. *Insect Biochem. Molec. Biol.*, 39(8): 516-522.

Zhang DH, Lax AR, Bland JM, Yu JJ, Fedorova N, Nierman WC, 2010. Hydrolysis of filter-paper cellulose to glucose by two recombinant endogenous glycosyl hydrolases of *Coptotermes formosanus*. *Insect Sci.*, 17(3): 245-252.

黄肢散白蚁 β-糖苷酶基因的克隆及特性分析

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摘要：【目的】黄肢散白蚁 *Reticulitermes flaviceps* 是我国代表性的食木白蚁之一，具有高效的纤维素酶系统。本研究旨在克隆黄肢散白蚁中纤维素酶基因并进行其特性分析，为白蚁纤维素酶资源的开发提供理论基础。【方法】采用反转录 PCR（RT-PCR）、简并 PCR 以及 cDNA 末端快速扩增实验（RACE）法克隆黄肢散白蚁 β-糖苷酶基因序列并进行生物信息学分析；利用大肠杆菌 *Escherichia coli* 原核表达该基因，表达出的蛋白质经纯化后进行纤维素酶活力测定。【结果】从黄肢散白蚁中克隆到一个 β-糖苷酶基因 *GZRfbg*1（GenBank 登录号：KU886065）的全长 cDNA 序列，全长 1 691 bp，开放阅读框 1 488 bp，编码 448 个氨基酸残基，预测分子量为 56.45 kD，具有典型的糖基水解酶家族 1（GHF1）的蛋白功能结构域。氨基酸序列多重比对显示，GZRfBG1 与北美散白蚁 *Reticulitermes flavipes* 的唾液腺 β-glucosidase 氨基酸序列一致性为 95%。GZRfBG1 在大肠杆菌 Rosetta 2（DE3）中进行体外表达，经纯化的 GZRfBG1 纤维素酶活力测定结果显示，其对水杨苷、羧甲基纤维素钠（CMC）以及纤维二糖的水解活性依次上升，能够在 CMC-琼脂平板中产生清晰的水解圈。【结论】结果表明，来自黄肢散白蚁与北美散白蚁两个近缘白蚁的唾液腺 β-糖苷酶在氨基酸序列及其水解特征方面亦具有高度保守性。该 β-糖苷酶基因的研究丰富了白蚁纤维素酶开发及系统发生研究的理论基础。

关键词：黄肢散白蚁；β-糖苷酶；cDNA；异源表达；唾液腺

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